



# High Resolution Mass Spectrometry in Combination with Capillary Electrophoresis as a tool for Metabolome Research

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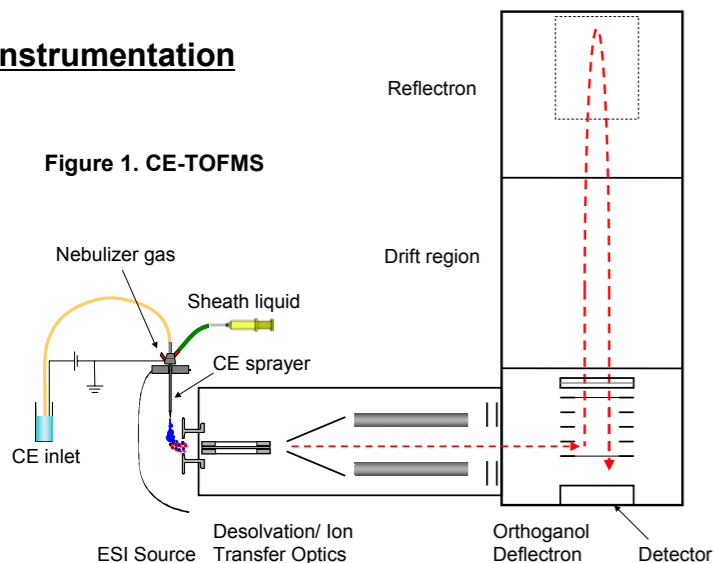


## Abstract

The study of global metabolite profiles (metabolomics) provides a holistic view of cellular metabolism. These profiles can be represented by analytical spectra obtained from high throughput methods. Herein, we intend to show the use of capillary electrophoresis coupled to high resolution mass spectrometry, such as TOFMS and FTMS to identify metabolites from *Shewanella Oneidensis*. This approach has the potential to be used as an effective tool in metabolome research for the characterization of metabolite profiles from organisms such as *S. Oneidensis*, a facultative aerobe proposed as a candidate for bioremediation of sites contaminated by heavy metals.

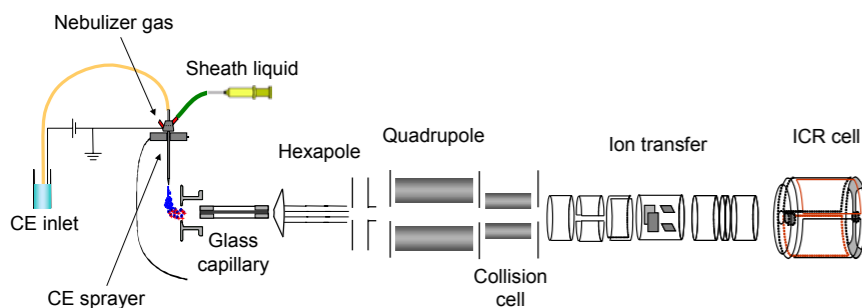
## Instrumentation

Figure 1. CE-TOFMS



A HP3D CE system coupled to a Bruker MicroTOF

Figure 2. CE-FTMS



A HP3D CE system coupled to a Bruker Apex Qe 7T FTMS

## Sample Preparation

A *S. Oneidensis* culture medium of 50 mL (with an optical density of 0.5) was subjected to methanol extraction, ultra-filtrated, lyophilized and reconstituted in 100µL HPLC grade water (in 10 % of the background electrolyte).

## CE-MS conditions

- Separations were conducted in a 1 meter, 40 µm i.d. fused silica capillary.
- 1.0 M and 1.6 M formic acid were used as run electrolytes for CE-FTMS and CE-TOF-MS respectively.
- A large volume sample stacking procedure with an ammonia plug, post sample plug, was performed at +30 kV.
- An Agilent sheath liquid interface consisting of 60:40 isopropanol/water + 1% formic acid was utilized at a flow rate of 3 µL/min.
- External calibration was used for ESI.
- A trigger time of 35 µs and 4000 averages were used for CE-TOFMS at an average resolving power of 10,000, whereas an average resolving power of 50,000 was used for FTMS.

## Results

With an unparallelled scan speed (acquisition rate), improved ion collection efficiency, extended mass range and accurate mass measurements, TOFMS is a useful tool in modern analytical laboratories. These attributes make the TOF mass analyzer ideally suited for high speed/efficient CE separations. Furthermore, the grounding of the sheath liquid interface together with the orthogonal arrangement of the CE probe, with respect to the MS capillary, ensures a robust and sensitive coupling.

Table 1. Regression Coefficients for Amino Acids via CE-TOF

Amino Acids	m/z	Coefficient (R <sup>2</sup> )
Gly	76	0.999992
Ala	90	0.999871
Ser	106	0.999918
L-Pro	116	0.999211
L-Val	118	0.999578
L-Thr	120	0.999477
L-Ile	132	0.999982
L-Leu	132	0.999982
L-Asp	134	0.999969
L-Lys	147	0.999986
L-Glu	148	0.999958
L-Met	150	0.999818
L-His	156	0.999948
L-Phe	166	0.999889
L-Arg	175	0.999987
Tyr	182	0.999809

Calibration curves at five concentrations, from 50 nM to 250 µM, were obtained for amino acid standards (Table 1). Regression coefficients for all amino acid standards were in all cases > 0.999 showing the quantitative power of CE-TOFMS. An excellent linearity of 3-4 orders of magnitude was observed for all compounds. Limits of detection below 50 nM were observed for most compounds (with a signal-to-noise >3). CE-TOFMS was then applied to metabolites extracted from *S. Oneidensis*.

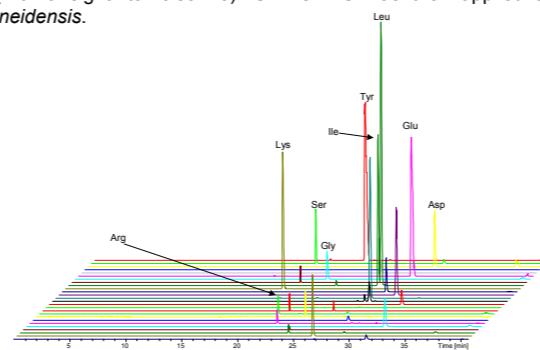


Figure 3. Extracted Ion Mass Electropherogram of *S. Oneidensis* via CE-TOFMS

Figure 3 shows the clear identification of various amino acids by CE-TOFMS. Mass accuracies of < 5 ppm were observed for all compounds (not shown). The high sensitivity exhibited by TOFMS ensures the detection of metabolites with low ionization efficiencies such as glycine. This feature of the TOF mass analyzer becomes advantageous when trying to capture the entire metabolome. In addition to TOFMS, the higher resolving power and mass accuracy of FTMS has been utilized with highly efficient CE separations to positively distinguish between compounds of the same nominal mass with a higher degree of confidence.

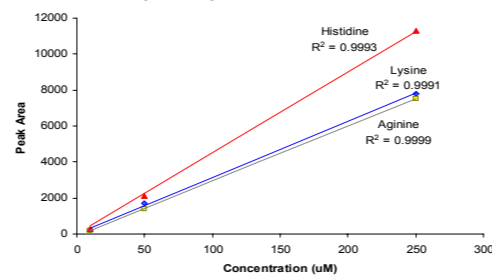


Figure 4. Calibration Curves for Histidine, Lysine and Arginine via CE-FTMS

Excellent linearity was obtained for selected amino acids at concentrations 10, 50 and 250 µM (figure 4) by CE-FTMS. Regression coefficients of > 0.999 were found in all cases. Thus CE-FTMS has the potential to be a useful quantitative tool.

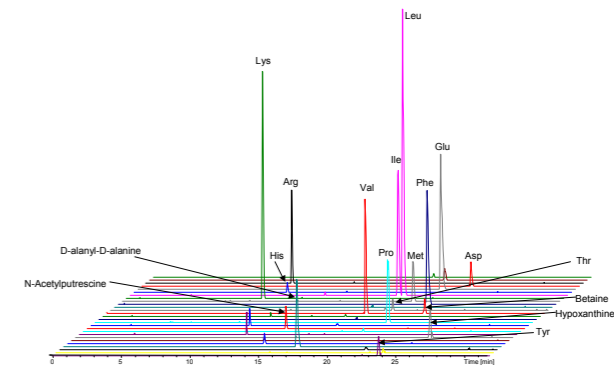


Figure 5. Extracted ion mass Electropherogram of *Shewanella Oneidensis* via CE-FTMS

Figure 5 shows the unambiguous identification of amino acids from a *S. Oneidensis* extract using migration times and exact mass measurements. Mass accuracies of < 2 ppm were observed for all compounds (table 2). However, a thorough screening process could yield even larger numbers of metabolites.

Table 2. Accurate mass measurements of targeted metabolites in *S. Oneidensis* via CE-FTMS

Amino Acid	Measured Mass	Theoretical Exact Mass	Error [ppm]	Concentration [µM]
Glycine	not detected	76.03931	-	not detected
Alanine	90.0549	90.05496	0.67	20.00
Serine	not detected	106.04987	-	not detected
Proline	116.07061	116.07061	0	4.41
Valine	118.08615	118.08626	0.93	6.98
2-Amino butyric acid*	118.08616	118.08626	0.85	present
Threonine	120.06553	120.06552	-0.08	0.87
Cysteine	not detected	122.02703	-	not detected
Isoleucine	132.10189	132.10191	0.15	2.22
Leucine	132.10188	132.10191	0.23	3.62
Asparagine	not detected	133.06077	-	not detected
Aspartic acid	134.04477	134.04479	0.15	4.00
Glutamine	147.07638	147.07642	0.27	present
Lysine	147.1128	147.11281	0.07	5.58
Glutamic acid	148.06032	148.06044	0.81	13.96
Methionine	150.05844	150.05833	-0.73	1.61
Histidine	156.07663	156.07676	0.83	0.36
Phenylalanine	166.08599	166.08626	1.63	2.31
Arginine	175.11899	175.11896	-0.17	2.22
Tyrosine	not detected	182.08117	1.69	present
Tryptophan	not detected	205.09715	-	not detected
D-alanyl-D-alanine*	161.09218	161.09207	-0.68	present
Cystine	not detected	242.03895	-	not detected
Acetylputrescine*	131.11779	131.11789	0.761	present
Betaine*	18.08615	118.08626	0.853	present
Hypoxanthine*	137.04579	137.04579	-0.042	present

\* Identified based on mass accuracies and empirical formula generation.

## Conclusions

- Excellent separation was obtained for both CE-TOF MS & CE-FTMS .
- Excellent linearity was obtained with both approaches.
- A good dynamic range was demonstrated by both CE-TOF-MS and CE-FT-MS.
- LOD's below 50 nM were observed for CE-TOF MS.
- Unambiguous identification of amino acids from *S. Oneidensis* extract was achieved by both CE-TOFMS & CE-FTMS.
- Mass errors < 5ppm and <2ppm were observed for CE-TOF and CE-FTMS respectively.

## Acknowledgements

- Bruker Daltonics for their collaboration on this project.
- This work is part of GTL (Genomes To Life), project funded by the Department of Energy.